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14. ABSTRACT

The goal of this project is to engineer T-cells for adoptive cell therapy of prostate cancer, by conjugating drug-loaded nanoparticles to therapeutic cells to enable targeted drug delivery to tumor sites and lymph nodes. Nanoparticles with cytokine proteins bound to their surfaces will be attached to the surface of T-cells— the equivalent of delivering a drug-loaded pill to each individual anti-tumor T-cell. These drug-carrying 'T-Pharmacytes' will be continuously stimulated by the protein and carry these cytokine-loaded particles wherever they traffic in the body. We hypothesize that this strategy will provide a safe and effective means to augment adoptive cell therapy and allow the great promise of this immunotherapy to be realized for treatment of prostate cancer. The proposed studies will provide a preclinical test of this concept. If successful, this approach to enhancing an immunotherapy strategy already in clinical trials might be translated to patient treatment in a relatively short timespan.

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None provided.

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T-Pharmacytes for Prostate Cancer Immunotherapy

Progress Report
Jianzhu Chen
May 2012

In this Synergistic Idea Development Award, the Chen group has been contributing to Specific Aim #3: *In vivo* antitumor responses elicited by nanoparticle-modified T-cells. For this purpose, it is necessary to monitor tumor size in live mice over time by live mouse imaging. Current imaging of prostate tumors in mouse models rely on genetically manipulated cell lines or imprecise quantification of autochthonous tumor growth. Introduction of fluorescent/luminescent proteins into pre-existing prostate cell lines and their subsequent surgical implantation does not faithfully mimic the natural progression of prostate tumor in situ. Analysis of the existing autochthonous prostate tumor mouse models requires MRI, which is costly and technically difficult, and *ex vivo* weight analysis of prostate tissue, which is not compatible with continuous monitoring of tumor growth.

To improve upon these systems, we have developed an inducible prostate cancer model in mice. Development of this model addresses the deficiencies in current prostate tumor models through the ability to: 1) track tumor growth by quantitative *in vivo* imaging, 2) assess endogenous tumor specific T cells using defined antigens, 3) delete specified genes known to effect prostate tumor growth in humans, and 4) dissect the effect of the endogenous immune response to tumors. Development of this new model system will facilitate the *in vivo* analysis of anti-tumor responses by nanoparticle-modified T cells.

In our new system (Figure 1), tumor formation is initiated in double transgenic $PTEN^{fl/fl}$ and $p53^{fl/fl}$ mice through injection of lentivirus expressing Cre recombinase into the prostate. To introduce T cell epitopes, the lentiviral vector also expresses the CD8 epitope SIYRYYGL (SIY), the CD8 epitope SIINFEKL (OVA_{257-64}) and the CD4 epitope ISQAVHAAHAEINEAGR ($OVA_{323-339}$). Control virus contains Cre-recombinase but lack model antigens. In addition, the lentiviral vectors express luciferase so that tumor growth can be imaged in live mice.

Our recent work has focused on characterizing this novel prostate cancer model. The injection of lentivirus into $PTEN^{fl/fl}/p53^{fl/fl}$ mice induces tumors of prostate origin. These tumors exhibit an aggressive phenotype characterized by invasion into the surrounding genitourinary tract tissue. Furthermore, imaging of luciferase signal from the prostates of these mice is feasible (Figure 2). We have assessed tumor development longitudinally from individual mice by quantitation of the luciferase signal. The increase in luciferase signal from the prostate coincides with the presence of aggressive prostate tumors. Current experiments are comparing the effect of tumor immunogenicity on prostate tumor development, through the use of vectors expressing or lacking the CD8 and CD4 epitopes described above. This model will provide an important tool for determining the effect of tumor specific nanoparticle-modified T cells on long term tumor growth.

In another study, we have investigated the role of co-stimulatory molecules in regulating functionality of tumor infiltrating CD8 T cells. A major obstacle to efficacious T cell-based cancer immunotherapy is the tolerizing tumor microenvironment that rapidly inactivates tumor-infiltrating lymphocytes. In an autochthonous model of prostate cancer (a modified TRAMP model), we have previously shown that intratumoral injection of antigen loaded dendritic cells (DCs) delays T cell tolerance induction as well as refunctionalizes already tolerized T cells in the tumor tissue. Now, we have defined molecular interactions that mediate DCs' effects. We show that pretreating antigen-loaded DCs with anti-CD70 antibody abolishes DCs' ability to delay tumor-mediated T cell tolerance induction, whereas interfering with 4-1BBL, CD80, CD86 or both CD80 and CD86 had no significant effect. In contrast, $CD80^{-/-}$ or $CD80^{-/-}CD86^{-/-}$ DCs failed to reactivate already-tolerized T cells in the tumor tissue, whereas interfering with CD70 and 4-1BBL had no effect. Furthermore, despite a high level of PD-1 expression by tumor infiltrating T cells and PD-L1 expression in the prostate, disrupting PD-1/PD-L1 interaction did not enhance T cell function in this model. These findings reveal dynamic requirements for costimulatory signals to overcome tumor induced tolerance and whave significant implications for developing more effective cancer immunotherapies, including the use of nanoparticle-modified T cells.

S. Peter Bak, Mike Stein Barnkob, Ailin Bai, Eileen M. Higham, K. Dane Wittrup, Jianzhu Chen. Differential Requirement for CD70 and CD80/CD86 in Dendritic Cell-mediated Activation of Tumor Tolerized CD8 T Cells. Revised for Journal of Immunology.

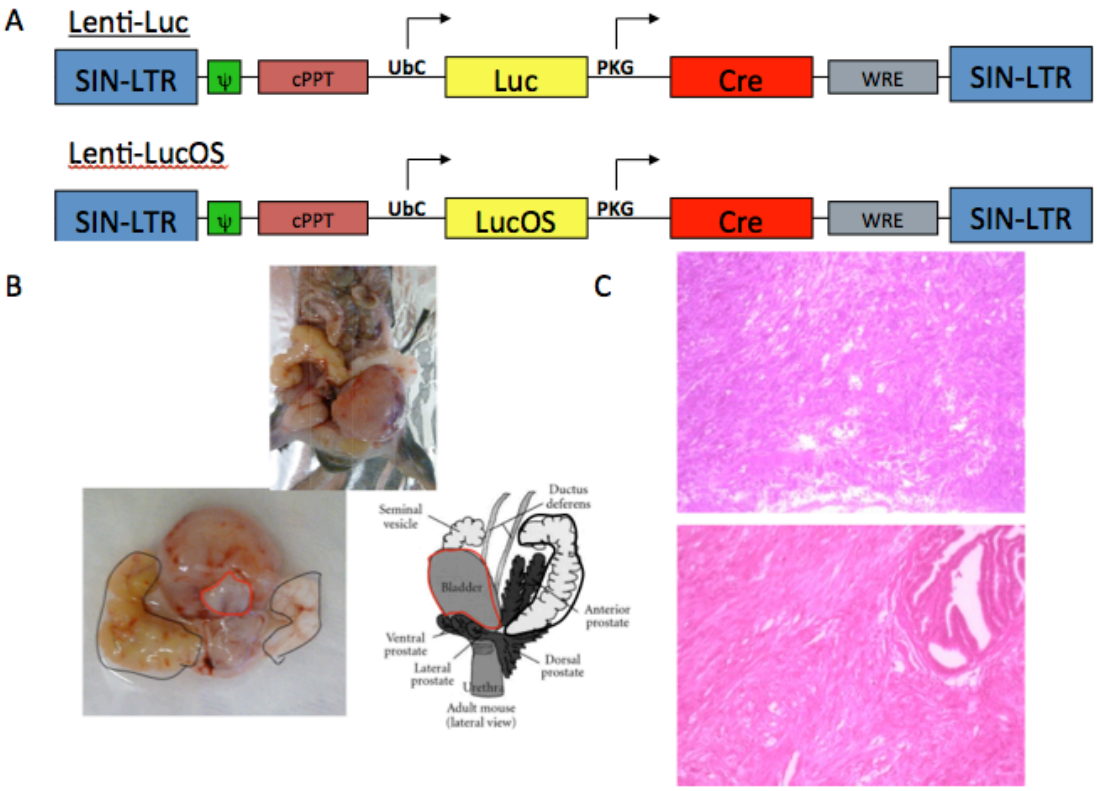

Figure 1. A. Schematic diagrams of the antigenic and non-antigenic lentiviral vectors. B. Representative tumor mass generated from lentivirus injection into PTEN^{flox}p53^{flox} mice. C. Representative H&E section from tumor mass, demonstrating the undifferentiated, aggressive phenotype.

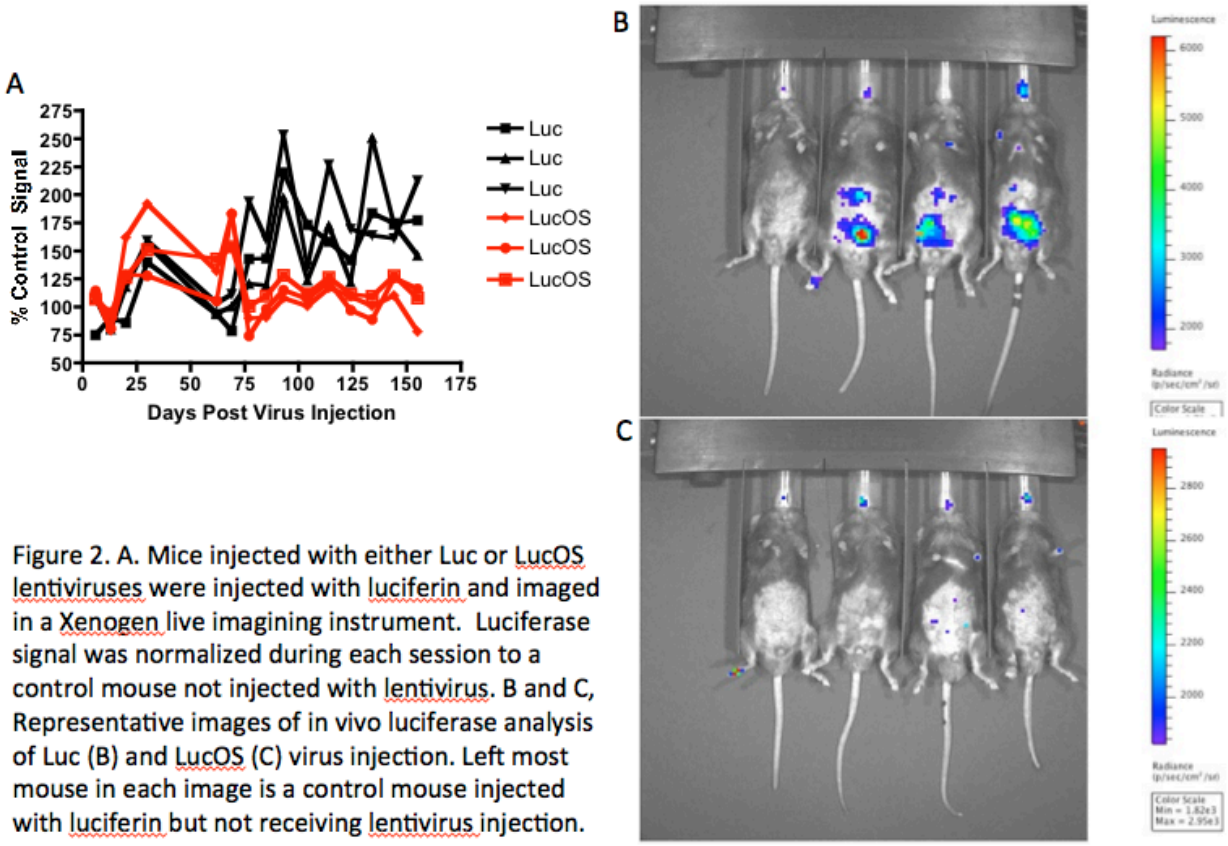

Figure 2. A. Mice injected with either Luc or LucOS lentiviruses were injected with luciferin and imaged in a Xenogen live imagining instrument. Luciferase signal was normalized during each session to a control mouse not injected with lentivirus. B and C, Representative images of in vivo luciferase analysis of Luc (B) and LucOS (C) virus injection. Left most mouse in each image is a control mouse injected with luciferin but not receiving lentivirus injection.